

## MODEL SYSTEM STUDIES WITH A PHASE SEPARATED MEMBRANE BIOREACTOR

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## ABSTRACT

The operation and evaluation of a bioreactor designed for high intensity oxygen transfer in a microgravity environment is described. The reactor itself consists of a zero headspace liquid phase separated from the air supply by a long length of silicone rubber tubing through which the oxygen diffuses in and the carbon dioxide diffuses out. Mass transfer studies show that the oxygen is film diffusion controlled both externally and internally to the tubing and not by diffusion across the tube walls. Methods of upgrading the design to eliminate these resistances are proposed. Cell growth was obtained in the fermenter using *Saccharomyces cerevisiae* showing that this concept is capable of sustaining cell growth in the terrestrial simulation.

## INTRODUCTION

The use of a bioreactor as a fermenter in Controlled Ecological Life Support Systems (CELSS) will likely occur in the food production or waste processing subsystems. It is anticipated that a design for a fermenter for an operational CELSS will be developed from models flown and tested on STS missions.

Probable areas of use

There are three possible places a CELSS-type bioreactor could be used:

i. As redundancy or backup for the conventional food production systems that would be available in space. It is clear that several systems could be developed, probably using plants and/or animals. However there is always the problem of catastrophic crop failure and if there is not enough stored food and it would be necessary to activate emergency rations of food. One possible source of this is microbial food which can be made available in two or three days. We have done preliminary studies that show that in reasonable sized fermenters it is possible to produce adequate quantities of edible types of biomass, for example yeast, that can be processed into the necessary food components.

ii. As supplements to conventional food production. The limiting amino acids for human nutrition are tryptophan and lysine. One of the deficiencies

in human foods such as wheat and similar materials is very easily satisfied by microbial sources. Many bacteria, and some yeasts, could provide the necessary amounts of lysine, methionine and tryptophan. This is just one example of a supplement and others may be possible. Also an analysis of human food balances reveals that even when using wheat and high quality foods humans are still short of carbohydrate. It is possible that it will always be necessary to have some calories from microbial carbohydrates.

iii. The area that will probably have first application for the bioreactor is the production of valuable commodities (in this case, food) from inedible plant waste. It is a consistent observation for all plants that about 50% of biomass is inedible. Of the inedible biomass, about 40% is comprised of cellulosics and about 20-30% is found in hemicellulosics (pentose sugars). These two components are readily separated with mild hydrolysis and fractionation methods. The further hydrolysis of these components into monosaccharides suitable for direct use or fermentation by microorganisms provides additional food sources for CELSS food production subsystems.

#### APPARATUS AND METHODS

The main problem with carrying out fermentations in microgravity is of course that the bubbles will not rise in the fermenter thus preventing gas - liquid disengagement (1). One reasonable solution is to avoid the need to solve the separation problem by not having a gas phase to disengage. The apparatus is designed to explore this concept for high-rate oxygen-transfer intensive microbial growth in a CELSS environment.

The gas and liquid phases are kept separate when the reactor contains about 10% by volume of silicone tubing in a zero-headspace fermentation configuration and passing the gas (air or oxygen) through the inside of the tubes. Oxygen and carbon dioxide are highly permeable to silicone rubber and diffuse rapidly through it. It is also possible to have liquid silicones saturated with oxygen passing through the tubes to act as oxygen carriers. Carbon dioxide can be readily removed from the off-gasses by adsorption in a sink such as monoethanolamine. A potentially attractive alternative to a fixed  $\text{CO}_2$  sink is reversible adsorption by redox-switched absorbers such as substituted metallocenes and quinones (2).

Such a system is essentially gravity-independent and can be readily examined under terrestrial conditions.

The terrestrial model tested was constructed from plexiglass in the form of a cylinder containing a total of 8.7 liters volume. The working volume was about 7.7 liters, the other liter being occupied by tubing and support frames. Thus 88% was available for culture.

150 feet of silicone tubing was wound round a support frame. The tubing had an internal diameter of 0.104 inches and an external diameter of 0.192 inches.

Stirring was provided to the center of the liquid by a marine impeller revolving at 200-400 revolutions per minute. Air flow to the inside of the tube could be varied by a mass flow controller from 2.5 to 20 liter per minute gas flow at an applied pressure of between 3 and 10 psig.

A 1.5% inoculum of *Saccharomyces cerevisiae* PEP4 was added to a synthetic medium (Yeast carbon base- YCB) supplemented with YM (1%) and 0.1% tryptone.

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100 ml of an overnight culture of the yeast are added. The head space is removed by adding enough YM broth to fill up the reactor, and then all the probes are inserted.

Oxygen transfer measurements were made by degassing the fermenter with nitrogen and following the rise of dissolved oxygen on a chart recorder as air was reintroduced through the tubes. Measurements were made with a New Brunswick galvanic oxygen probe.

## RESULTS & DISCUSSION

### Yeast growth

In order to evaluate the reactor under actual growth conditions, cultures of yeast were grown under a variety of reactor conditions. Figure 1 was an initial run at low gas pressure but high flow rate. There was no attempt to control pH or temperature. The data showed us that the apparatus and sterilization techniques could be employed to culture yeast cells. The effect of lowering the flow rate by one half and increasing the pressure is shown in figure 2. Again, the system worked well and the rate of cell growth increased as is shown by the quicker depletion of oxygen (20 hours vs. 30 hours).

Since oxygen was apparently supplied at adequate levels, an attempt was made to evaluate the lower working point of the apparatus. The time at which oxygen depletion occurred as a function of reactor conditions was used as the basis for evaluating the lower working limit of the apparatus. The initial experiment in this series is shown in figure 2. With only 50 feet of tubing, 7.5 psig. and 1 liter/min flow rate, the reactor reached oxygen depletion after about 14-15 hours. However, the possibility that glucose depletion was the cause for lowered oxygen consumption could not be ruled out. In the experiment shown in Figure 3, the flow rate was lowered even more and the glucose measurements were taken more frequently. The results showed that glucose depletion had not occurred simultaneously with oxygen depletion. This indicates that the cells are growing at a rate that was a direct function of oxygen supply. The other observation was that by lowering the flow rate to 0.5 liter/min, the point at which oxygen was depleted was shifted to 16-17 hours. This is slightly higher than the value shown in Figure 2 and is consistent with the fact that airflow was half that of the value used in the Figure 2 experiment. The cells are still healthy and normal. The maximum cell count is 1.3 grams per liter. This is not a high density, but it is encouraging for our first design.

These simple experiments showed the following:

1. The reactor could be sterilized, operated and maintained using the simple equipment employed (i.e. no temperature or pH control) to provide meaningful results.
2. Oxygen limitation can be reached in a relatively short time permitting quick analysis of the system.
3. Measurements of oxygen transfer rates will need to be conducted in order to estimate actual maximum operating limits.

### Oxygen transfer studies

The oxygen transfer data from the step response studies were analyzed by the method of Ruchti et al. (3), and expressed as the product of the overall mass transfer coefficient and the surface area per unit volume of reactor,  $K_{la}$ . The measurements were taken for a range of air flow rates and stirrer speeds and are given in Table 1.

The biological experiments demonstrated that modest cell dry weights could be obtained with this design of fermenter before oxygen limitation was reached. While these results are encouraging they clearly are not adequate for a practical system. To overcome the inherent limitation of the preliminary equipment design, the oxygen transfer studies were initiated. The values of  $K_{la}$  obtained were some 50-100 times lower than in conventional stirred fermenters operating under terrestrial conditions. They correspond to oxygen transfer intensities of around 0.04 kg O<sub>2</sub>/m<sup>3</sup>/hr.

Three main effects can be expected to contribute to the low oxygen transfer intensities observed in this study:-

- a. film diffusion resistance in the tube containing the gas
- b. external film diffusion into the bulk liquid
- c. oxygen diffusion across the silicone tubing wall

For laminar flow of the gas and liquid, resistances a and b above will be reduced as the flow rate past the tubes is increased while resistance c will be unchanged. From fluid mechanics it is known that the mass transfer coefficient will vary inversely with the square root of the flow rate. A common way of therefore assessing the relative importance of the contributions is to plot the reciprocal of  $K_{la}$  vs. the reciprocal of the square root of the flow rate, extrapolate to zero on the axis, i.e., infinite velocity which removes the film resistance and compare the magnitude of the residual mass transfer coefficient (4).

$$\frac{1}{K_{la}} = \frac{1}{(velocity, internal)^{1/2}}$$

$$+ \frac{1}{(velocity, external)^{1/2}}$$

$$+ (membrane diffusion resistance) \dots \dots \dots (1)$$

Figure 4 shows this procedure for the internal flow rate variation experiment. The graph shows a marked slope implying that indeed the internal diffusion resistance in the tube is substantial and that major improvements in oxygen transfer can be expected simply by increasing the flow rate, perhaps with recycle, through the tubes.

The residual mass transfer resistances can now be subtracted out and the effect of external film resistances examined. Figure 5 shows the same kind of graph, this time produced by changing the stirrer speed. Again a substantial slope is observed with the regression line passing through the origin of the graph, i.e., at infinite stirrer speed the mass transfer coefficient becomes infinite. The interpretation of this is that the external fluid resistances are extremely high compared to which any resistance from the oxygen diffusion across the membrane is negligible.

These results are very reassuring as they imply that redesign of the equipment can be done in ways that will result in very substantial increases in oxygen transfer efficiency that will permit large increases in cell mass to be obtained long before the diffusion resistances in the tubes themselves start to become important.

The reactor will be reconfigured to reflect these findings.

#### CONCLUSIONS

1. Yeast can be successfully grown in a phase separated fermenter that should be capable of operation independent of gravity.
2. The current design limitations can be overcome and will result in substantial increases in oxygen transfer intensities which in turn will support greater cell masses to provide a practical test facility for a CELSS test bed.

**TABLE 1** Mass transfer coefficients ( $K_{1a}$ ) as a function of system variables.

Airflow Rate (lit/min)	Stirrer Speed (rpm)	$K_{1a}$ ( $hr^{-1}$ )
9.5	325	4.20
7.5	325	2.75
5.0	325	2.64
2.5	325	2.08
7.5	275	3.47
7.5	275	2.75
7.5	225	2.77
7.5	110	1.97
7.5	155	1.29

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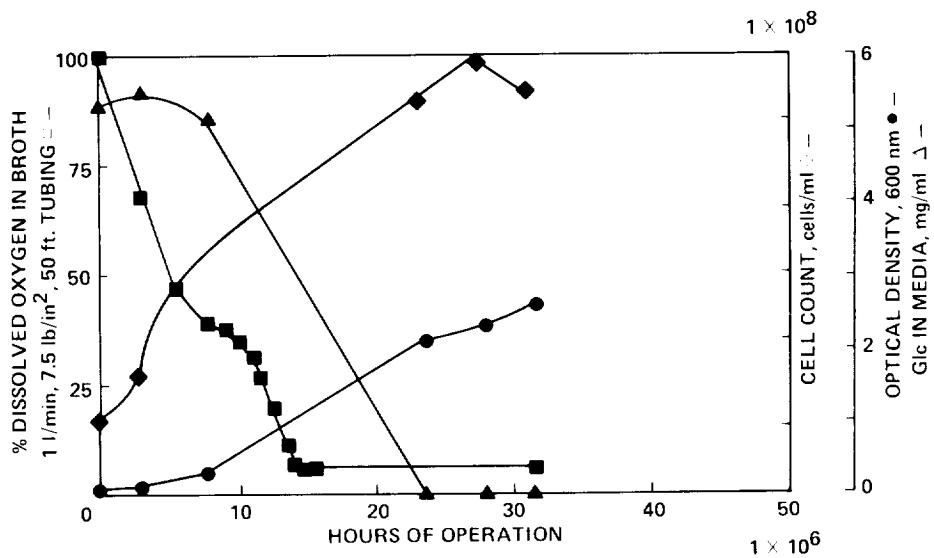


Fig. 1. Initial evaluation of the fermenter. The lowest working point of the fermenter was established. With only 50 feet of tubing, 7.5 psig, and 1 liter/min flow rate the reactor reached oxygen depletion after about 14-15 hours.

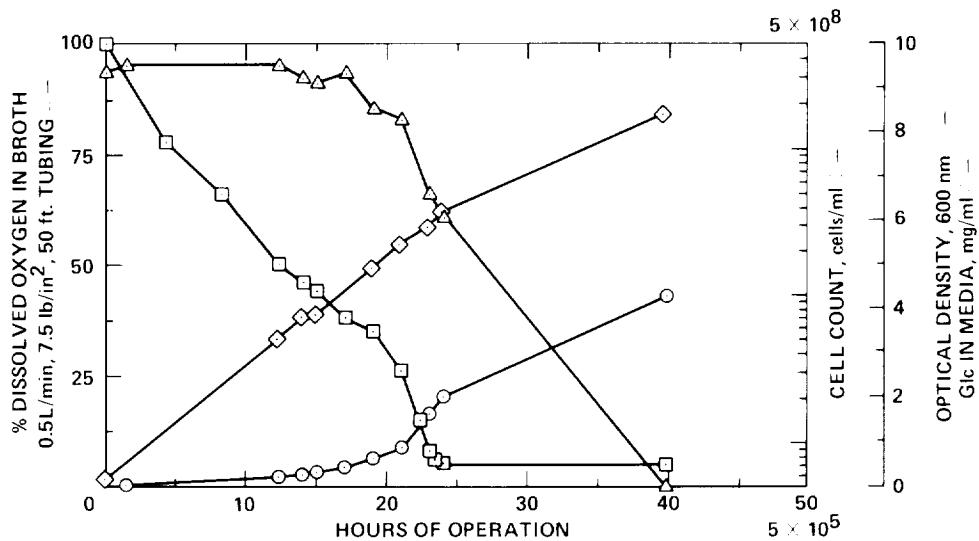


Fig. 2. At even lower flow rate glucose depletion does not occur simultaneously with oxygen depletion, indicating that the cells are growing at a rate that was a direct function of oxygen supply.

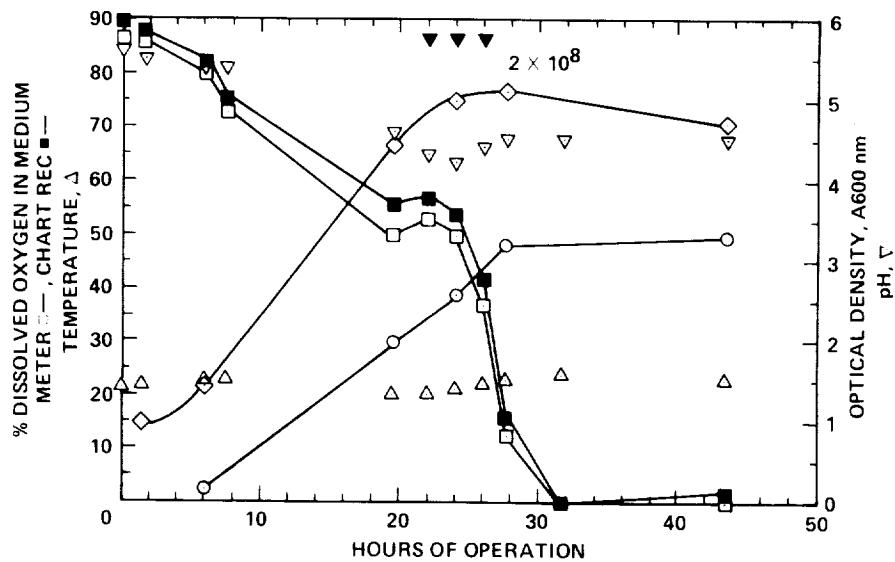


Fig. 3. Further reduction in the flow rate shows that the cells were growing at a rate that was a direct function of the oxygen supply. Thus oxygen will remain the limiting nutrient in these experiments.

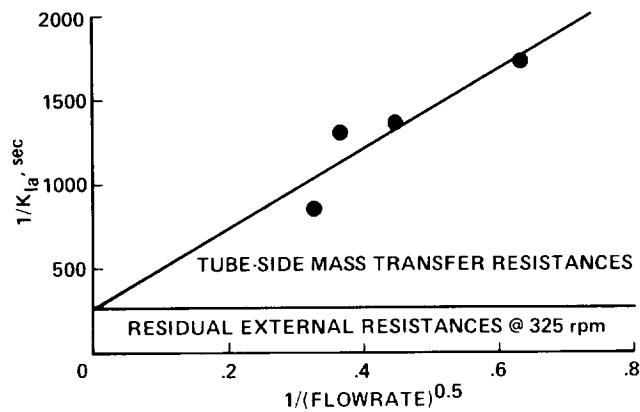


Fig. 4. The procedure for assessing the internal flow rate resistances. The graph shows a marked slope implying that indeed the internal diffusion resistance in the tube is substantial and that major improvements in oxygen transfer can be expected simply by increasing the flow rate.

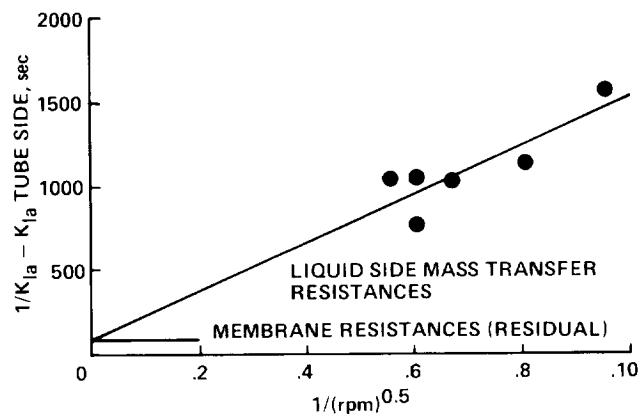


Fig. 5. The residual mass transfer resistances from Figure 4 are subtracted out and the effect of external film resistances examined by changing the stirrer speed. Again a substantial slope is observed with the regression line passing near the origin of the graph, i.e. at infinite stirrer speed the mass transfer coefficient becomes infinite, implying that the external fluid resistances are extremely high compared to which any resistance from the oxygen diffusion across the membrane is negligible.

